pronase/collagenase digestion from the articular cartilage of the metatarso-phalangeal joints of 18 month old calves from a local slaughterhouse. The BPAC and the human chondrogenic cell line C28/I2 were cultured in micromass to promote differentiation and extracellular matrix production. For gain- and loss-of-function experiments, chondrocytes were transfected with a mammalian expression vector encoding for full-length Agrin (FL-Agrin) or with Agrin siRNA's respectively. Accumulation of cartilage-specific extracellular matrix production, rich in highly sulphated glycosaminoglycans (GAGs), was quantified by alcian blue staining at pH 0.2 and alizarin red staining. Chondrogenic potential was measured using pellet analysis as previously described3, BPAC pellets were cultured for 14 days, weighed, sectioned and stained with alizarin red and safranin o. Gene expression analysis of Agrin, Col2A1, Aggrecan, Sox9 was performed by real time PCR. Immunofluorescence was used to determine the expression levels of Agrin at protein level in chondrocytes cultured in monoloayer and in paraffin-embedded tissue sections.

Results: Agrin was expressed in healthy adult human and bovine articular cartilage. Agrin was signifcantly downregulated in human osteoarthritic cartilage. This downregulation was also replicated in experimental murine osteoarthritis, indicating that it is a consequence rather than a cause of osteoarthritis. Agrin expression was partially retained in the articular cartilage of sham operated knees, indicating that inflammation alone (present both in DMM and in sham) is not sufficient to induce Agrin downregulation, but cartilage damage is necessary. Endogenous expression of Agrin was confirmed in C28/I2 and in BPAC. Overexpression of FL-Agrin resulted in enhanced differentiation as demonstrated by upregulated the cartilage key transcription factor Sox9. . Knock-down of Agrin by siRNA resulted in reduced GAG production in C28/I2 and chondrocyte de-differentiation as documented by decreased expression Sox9, Col2A1 and Aggrecan mRNA.

Conclusions: Agrin expressed in healthy adult articular cartilage and is downregulated in osteoarthritis; - Agrin is anabolic in cultured articular chondrocytes; - Agrin is required for chondrocyte differentiation in vitro.

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AGE- AND SEX-DEPENDENCE OF FEMOROTIBIAL CARTILAGE CHANGE AFTER ANTERIOR CRUCIATE LIGAMENT (ACL) TEAR – 5 YEAR FOLLOW UP IN THE KANON STUDY

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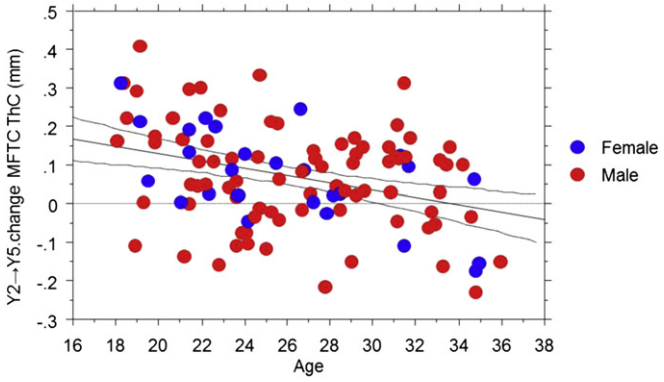
Purpose: Anterior cruciate ligament (ACL) tear is known to increase the risk of OA, and is associated with acute joint trauma and chronically altered joint mechanics. An increase in medial femorotibial cartilage thickness (ThC) has been described within 1-2 years after ACL tear. However, whether this increase depends on age or sex, whether it represents an early pathological event (caused by trauma), and/or whether it persists (due to a chronic alteration in joint mechanics) is unclear. Hence, we studied ThC change between 2 to 5 years follow-up (Y2→Y5) and between baseline and 2 years (BL→Y2).

Methods: 121 young active adults with an acute ACL tear in a previously uninjured knee were included in a randomized control trial, comparing rehabilitation plus early ACL reconstruction (ACLR; n=62) with rehabilitation plus the option of delayed ACLR (n=59). Sagittal MRIs (3D/WATSc) were acquired within 5 weeks of the tear (BL), and at Y2 and Y5 (n=107; 81men, 26 women; median age 25.6y; age range 18-36). ThC in the medial (MFTC) and lateral (LFTC) compartment was measured after segmentation of femoral and tibial cartilages, with blinding to acquisition order and treatment group. Regression analysis (Pearson) and unpaired t-tests were used to explore the relationship of post-tear cartilage changes with age and sex.

Results: The increase in MFTC ThC from Y2→Y5 was +1.8% (mean±SD [95% CI]: +70±130μm [45, 95];) compared with +1.3% from BL→Y2 (+49±165μm [17, 80]). The Y2→Y5 MFTC ThC increase did not differ significantly (p=0.94) between men (69±134μm; [40, 99]) and women (71±120μm; [23, 120]), but was significantly (p=0.017) greater in those younger than group median age of 25.6y (99±137μm [62, 137]) than in those older than group median (40±117μm [7, 72]). The correlation (r) of MFTC ThC change from Y2→Y5 with age (Fig. 1) was -0.35 [-0.51,-0.17]. For comparison it was -0.26 [-0.43,-0.07] for BL→Y2, and -0.44 [-0.58, -0.27] for BL→Y5. No significant increase in LFTC ThC was observed and no significant relationship of LFTC change with age. Baseline cartilage thickness in MFTC (but not LFTC) correlated positively with age in men (+0.27 [0.05; 0.46]) and women (+0.30 [-0.10; 0.62]). The annual increase in MFTC ThC (from age 18) estimated from the regression equations was +25μm/y in men and +22μm/y in women.

Conclusions: Our findings suggest that the MFTC ThC increase in young adults continues during Y2→Y5 after ACL tear. This increase is stronger in younger than in the more mature adults, with age explaining 12% of the Y2→Y5 and 19% of the BL→Y5 variability. The (baseline) cross sectional findings indicate that there may exist a physiological increase in MFTC ThC with age in early adulthood that correspond in magnitude with those observed after ACL tear, but longitudinal studies will have to confirm this hypothesis. Hence we recommend that young healthy controls be studied longitudinally to differentiate pathological ThC change after ACL tear from physiological maturation. Further, we recommend that analyses comparing ThC changes after early ACLR vs. the option of delayed ACLR adjust for age.



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SYNERGISTIC EFFECTS OF HYPOXIA AND BMP-2 ON THE HUMAN ARTICULAR CHONDROCYTE PHENOTYPE

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Articular cartilage has a very limited ability to regenerate spontaneously after injury. The unique cell type of that tissue (the chondrocytes) produce specific extracellular matrix components, which give to cartilage its biomechanical properties. Beside growth factors, oxygen concentration is considered as an important regulator of the matrix surrounding the chondrocytes. It is known that cartilage is a hypoxic tissue not only during development but also in adult cartilage. A growing number of evidences show that hypoxia influence several chondrocyte functions, but the exact effects and underlying mechanisms of hypoxia on the adult chondrocytes are not fully understood. In addition to explain how the chondrocyte phenotype is controlled, this study could be helpful to improve the cell based cartilage therapies.

Aim of the study: we previously showed that hypoxia, through a HIF-2α mediated pathway, up-regulates major chondrocyte markers, unknown chondrocyte-associated genes, and cartilage-specific miRNAs. Here we investigated the effect of hypoxia on the production of collagens by human articular chondrocytes, and if hypoxia could interfere with other chondrogenic factors such as BMP-2.

Methods: primary cultures of human articular chondrocytes were assessed for their ability to redifferentiate, under a treatment with the chondrogenic factor BMP-2, either in normoxia (20% O2) or hypoxia (1% O2).

Results: compared to normoxia, hypoxia environment dramatically enhanced the BMP-2 effect on Sox9 and type II collagen expressions.